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Supporting Information

Vibrational Imaging of Glucose Uptake Activity in Live Cells and Tissues by Stimulated Raman Scattering**

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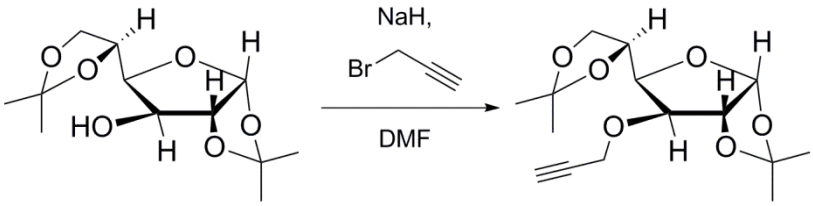
Supporting Information

Organic synthesis

Synthesis of 3-O-propargyl-D-glucose. Reagents and solvents were obtained from Sigma-Aldrich and were used without further purification. Flash chromatography was performed on a Teledyne ISCO CombiFlashRf using RediSepRf silica gel columns. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 (400 MHz) Fourier Transform (FT) NMR spectrometer at Columbia University, Chemistry Department. ^{1}H NMR spectra are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), number of protons. Chemical shifts are referenced to the solvent residual peak. Fast Atom Bombardment (FAB) high resolution mass spectra (HRMS) were recorded on a JMS-HX110A mass spectrometer.

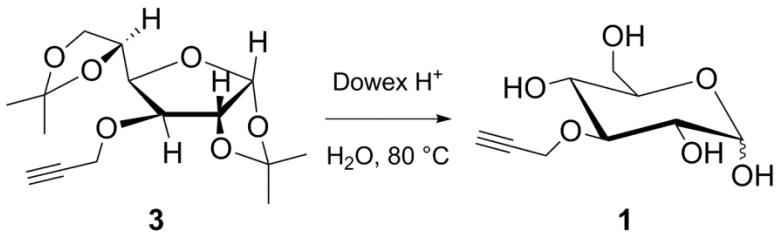


2 **3**

To a solution of 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (**2**, 500 mg, 1.92 mmol, Aldrich D7600) in 10 mL dry DMF was added sodium hydride (138 mg, 5.8 mmol) at 0 °C. The solution was stirred at 0 °C for 30 min before propargyl bromide (80% in toluene, 0.43 mL, 3.84 mmol) was added dropwise. The reaction was stirred at room temperature for 12 h before quenched with saturated ammonium chloride solution (10 mL). The mixture was extracted with ethyl acetate (2 * 25 mL), and the organic layer was combined, dried over anhydrous sodium sulfate, concentrated in vacuo, and purified by column chromatography on silica gel (0-50% Ethyl acetate in Hexanes) to give **3** (518 mg, 90%) as a colorless oil. The ^{1}H NMR spectrum is in accordance with previously published values (A. Hausherr et al., *Synthesis*, **2001**, 1377).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (d, *J* = 3.6 Hz, 1 H), 4.30 - 4.24 (m, 3 H), 4.14 (dd, *J* = 7.6, 2.8 Hz, 1 H), 4.11 - 4.06 (m, 2 H), 3.99 (dd, *J* = 8.8, 5.6 Hz, 1 H), 2.47 (t, *J* = 2.4 Hz, 1 H), 1.50 (s, 3 H), 1.42 (s, 3 H), 1.35 (s, 3 H), 1.31 (s, 3 H).

HRMS (FAB+) m/z Calcd. for $C_{15}H_{23}O_6$ $[M+H]^+$: 299.1495. Found: 299.1496



3 **1**

To **3** (594 mg, 1.99 mmol) was added water (10 mL) and Dowex® 50WX8 hydrogen form (600 mg, Sigma-Aldrich 217514). The mixture was heated to 80 °C for 20 h before filtered. The filtrate was concentrated *in vacuo* to give **1** (416 mg, 1.91 mmol, 96%) as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 5.13 (d, *J* = 3.6 Hz, 1 H), 4.44 (d, *J* = 2.4 Hz, 2 H), 3.79 – 3.73 (m, 2 H), 3.70 – 3.61 (m, 2 H), 3.51 (dd, *J* = 9.8, 3.8 Hz 1 H), 3.40 (t, *J* = 9.6 Hz, 1 H), 2.82 (s, 1 H). ^{13}C NMR (101 MHz, D_2O) δ 92.1, 80.8, 79.8, 75.9, 71.4, 71.2, 69.2, 60.4, 59.9.

HRMS (FAB+) m/z Calcd. for $C_9H_{14}O_6Na$ $[M+Na]^+$: 241.0688. Found: 241.0683

Cell culture and sample preparation

Materials. DMEM (11965), fetal bovine serum FBS (10082), penicillin-streptomycin (11540), Neurobasal-A medium (10888), Glucose-free Neurobasal-A medium (A24775), B27 serum-free supplement (17504) and L-glutamine (25030) are purchased from Invitrogen. Glucose-free DMEM (D5030), Phosphate buffered saline PBS (D8537 and D8662) and 2-NBDG (72987) are obtained from Sigma-Aldrich. Culture media for HeLa, L6 and U-87 MG cells is prepared by adding 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin into DMEM. Neuron culture media is prepared from Neurobasal-A medium added with 1× B27 serum-free supplement and 0.5 mM L-glutamine.

For tumor cell lines, cells are seeded on coverslips in 24-well plates each with ~0.5 mL of culture media and incubated at 37 °C and 5% CO_2 for 2 days before experiment. The culture media are then replaced with regular media without D-glucose and supplemented with 8 or 25 mM 3-OPG (from 250 mM stock solution in PBS) for various time at 37 °C and 5% CO_2.

For competition experiments with glucose, HeLa cells are incubated with 25 mM 3-OPG for 4 hours in the presence of 0, 10, 50 mM D-glucose or 50 mM L-glucose at 37 °C and 5% CO_2 before imaging.

For RNA interference experiments, ~60% confluent HeLa cells are forward transfected with 10 nM Silencer Select Negative Control No. 2 siRNA (Ambion 4390846) or 10 nM Silencer Select GLUT1 siRNA (s12925 validated, Ambion 4390824) using Lipofectamine RNAiMAX reagent (Invitrogen 13778) in regular media without antibiotic and after 48 hours, cells are incubated in glucose-free media with 25 mM 3-OPG for 30 minutes at 37 °C and 5% CO_2 before imaging.

For cytochalasin B inhibition experiments, HeLa cells are incubated in glucose-free media with 25 mM 3-OPG in the presence of 0.05% DMSO vehicle (control) or 10 μM cytochalasin B (Sigma C2743) for 30 minutes at 37 °C and 5% CO_2 before SRS imaging.

For insulin induced glucose uptake experiments, L6 cells are seeded on coverslips in 24-well plates and grown to confluence in regular DMEM media at 37 °C and 5% CO_2. The media are then changed to

DMEM with 2% horse serum (Sigma H1138) and 1% (v/v) penicillin-streptomycin for 2-3 days to induce differentiation and fusion into muscle cells. Then L6 muscle cells are incubated with or without 1 μg/ml insulin (Gibco 12585) in fresh low-serum media for 24 hours. After that, the media are changed to glucose-free media with 25 mM 3-OPG for 30 minutes at 37 ℃ and 5% CO_2 before imaging.

For 3-OPG efflux experiments, HeLa cells are first incubated in glucose-free media added with 25 mM 3-OPG for 30 minutes at 37 ℃ and 5% CO_2. After that, the media are changed to fresh glucose-free media with or without 10 μM cytochalasin B for 0, 30 and 60 minutes incubation at 37 ℃ and 5% CO_2 before imaging.

For uptake kinetics experiments, HeLa cells are incubated in glucose-free media with 25 mM 3-OPG for various time points at 37 ℃ or RT and 5% CO_2 before imaging.

For 3-OMG competition experiments, HeLa cells are incubated in glucose-free media with 25 mM 3-OPG in the absence or presence of 50 mM 3-OMG (Sigma M4879) for 30 minutes at 37 ℃ and 5% CO_2 before imaging.

For primary mouse hippocampal neurons, cells are seeded on coverslips in 24-well plates each with ~1 mL of neuron culture media and incubated at 37 ℃ and 5% CO_2 for 7 days. Then the media is changed to glucose-free neuron culture media supplemented with 1 mM sodium pyruvate and 3-OPG (250 mM in PBS solution) is added into the media with a final concentration of 32 mM for 1 hour.

After incubation of all cell samples, the media is discarded and the coverslip is washed gently with 1 mL PBS buffer twice, assembled into a chamber filled with PBS solution and ready for spontaneous Raman scattering measurement and SRS imaging.

For simultaneous fluorescence and SRS imaging of 2-NBDG and 3-O-propargyl-D-glucose uptake, HeLa cells are incubated in glucose-free media with 25 mM 3-OPG and 100 μM 2-NBDG for 1 hour. Cells are then washed with 1 mL PBS twice and assembled into a chamber filled with PBS solution for imaging. Fluorescence images are collected using an Olympus FV1200 confocal microscope with 488 nm laser excitation and 505-605 nm bandpass filter.

Cell viability assays are performed using the LIVE/DEAD viability/cytotoxicity kit for mammalian cells (Molecular Probes L-3224). In brief, HeLa cell standards and HeLa cells treated with or without 25 mM 3-OPG in glucose-free media for 4 hours are incubated with 2 μM calcein AM and 4 μM EthD-1 working solution for 20 minutes at 37 ℃ before imaging. Two-color fluorescence images are collected using an Olympus FV1200 confocal microscope with 488 nm laser excitation and 505-525 (calcein-AM) and 655-755 nm (EthD-1) bandpass filter sets.

Tissue culture and sample preparation

All mice experiments are performed with approval from Columbia University IACUC. Mouse tumor xenograft model is generated by subcutaneously injecting 5-10×10^6 U-87 MG human glioblastoma cells into the left flank of female J:NU immunodeficient mice (Jackson Laboratory) and allowed to grow for 3-4 weeks.

Ex vivo culture of mouse U-87 MG tumor xenograft slices. Tumors embedded in agarose are sliced with a Leica vibratome at 400 μm thickness and quickly transferred to Millicell cell culture insert (Millipore PICM03050) in DMEM media at 37 °C and 5% CO_2. After 1 day culture, the media is changed to DMEM media without glucose and 3-OPG (in PBS solution) is added into the media with a final concentration of 32 mM for 3 hours before imaging.

For *ex vivo* slice culture of mouse brain. 300 μm thick brain slices are obtained from D26 mice (C57BL/6J) using a Leica vibratome and plated on Millicell cell culture insert in glucose-free Neurobasal-A medium added with 1× B27 serum-free supplement, 1 mM L-glutamine and 1 mM sodium pyruvate. 3-OPG (in PBS solution) is added into the media with a final concentration of 32 mM for 3 hours at 37 °C and 5% CO_2 before imaging.

After incubation, the insert is taken out and the tissue is washed twice with 1 mL PBS buffer. The tissue slice is then transferred to a chamber filled with PBS solution and a coverslip is used to close the chamber for SRS imaging.

Stimulated Raman scattering microscopy

An integrated laser system (picoEMERALD, Applied Physics & Electronics, Inc.) is used to produce all laser beams. A 1064 nm Stokes beam with 6 ps pulse width is generated at 80 MHz repetition rate, and part of the Stokes beam is converted to 532 nm and used to seed an optical parametric oscillator to produce a picosecond mode-locked signal (pump beam) with 5-6 ps pulse width (the idler is blocked with an interferometric filter). The wavelength of pump beam is tunable from 720 to 990 nm. The rest of Stokes beam is sinusoidally modulated in intensity using a built-in electro-optic-modulator at 8 MHz with over 95% modulation depth. Two dichroic mirrors inside the laser system are used to adjust the spatial overlap of pump and Stokes beam and the temporal overlap is optimized using a built-in delay stage based on the heavy water SRS signal.

Both laser beams are introduced into an inverted multiphoton laser-scanning microscope (FV1200MPE, Olympus) which maximizes near IR output, and are focused onto the sample through a 25× water objective (XLPlan N, 1.05 N.A. MP, Olympus) with high transmission at near IR wavelength. The size of pump and Stokes beams is optimized to match with the back-aperture of the objective. After passing through the sample, a high N.A. condenser lens (oil immersion, 1.4 N.A., Olympus) is used to collect pump and Stokes beams with high efficacy in Kohler illumination. The laser-scanning motion is descanned using a telescope. The Stokes beam is blocked by a high O.D. bandpass filter (890/220 CARS, Chroma Technology) and only the pump beam is collected by a large area (1 cm × 1 cm) silicon photodiode (FDS1010, Thorlabs). The photodiode is reverse-biased with a 64 DC voltage to increase the saturation threshold and response bandwidth. After passing through a 8 MHz electronic bandpass filter (KR 2724, KR electronics) remove both high frequency laser pulsing component and low frequency fluctuations due to laser scanning, the generated current of photodiode enters a radio frequency lock-in amplifier (SR844, Stanford Research Systems) terminated with 50 Ω to demodulate the stimulated Raman loss signal from the pump beam. SRS images with 512 × 512 pixels per frame are generated by inputting the in-phase signal at the X channel of the lock-in amplifier to the analog interface box (FV10-ANALOG) of the microscope. ~150 mW modulated Stokes beam and ~140 mW pump beam, measured after the 25× water objective, are used to image the sample at all frequencies. The imaging pixel dwell time is 100 μs with ~26 s/frame and the time constant for demodulation is 30 μs.

Spontaneous Raman spectroscopy

Spontaneous Raman scattering spectra are acquired on an upright confocal Raman microscope (Xplora, Horiba Jobin Yvon) with 532 nm diode laser source and 1800 l/mm grating at room temperature. The

excitation power is ~27 mW after passing through a 100×, air objective (MPlan N, 0.90 N.A., Olympus) and 80 s acquisition time is used to collect Raman spectra of all samples at single point under identical condition. The water Raman background is subtracted from all spectra.

Image processing and data analysis

All images are assigned colors with ImageJ 1.48b. All data for comparisons are measured under identical imaging conditions. SRS image intensity of each experiment is calculated by summing the signal from all cells within the optical field divided by the total cell area using MATLAB R2012a. The values from multiple independent experiments are then averaged to give the mean and standard deviation for each condition. Data fitting is carried out with OriginPro 8. Two-tailed student's *t*-tests are performed with online calculator (GraphPad).

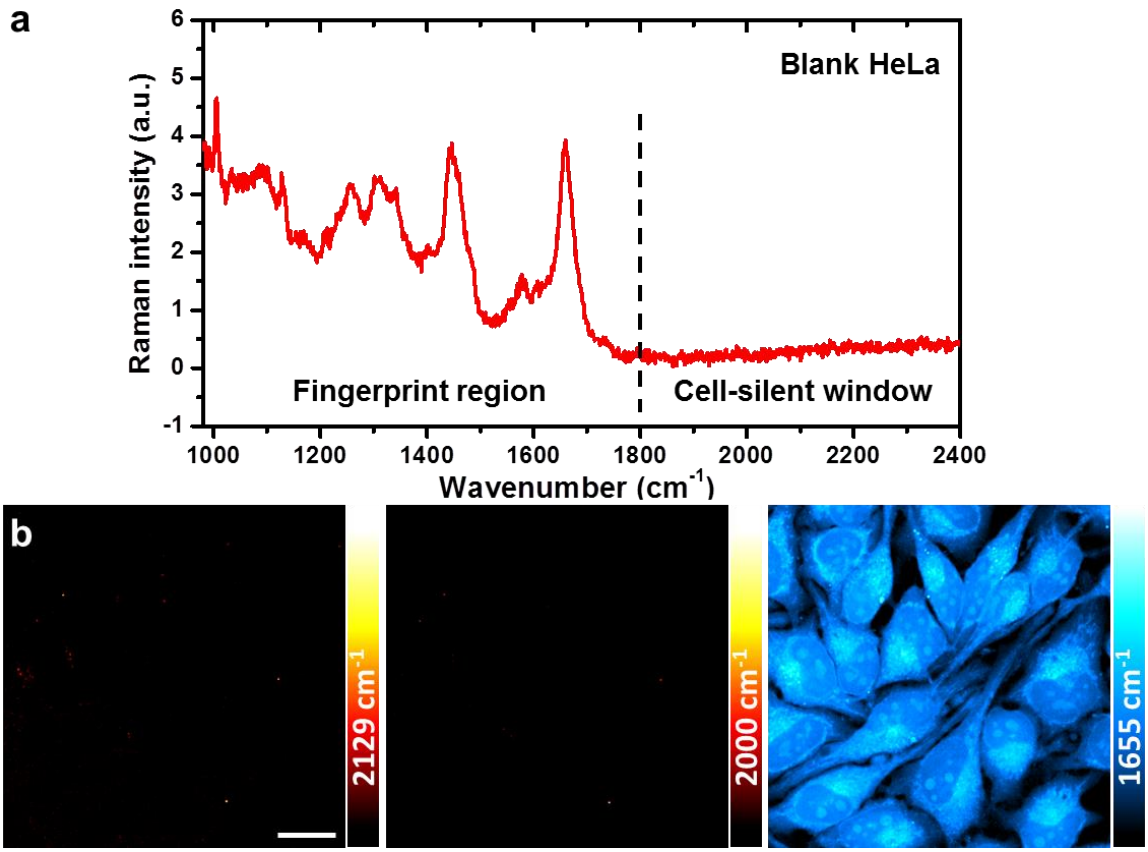


Figure S1. Raman background of live mammalian cells. (a) Spontaneous Raman spectrum of blank live HeLa cells displays a flat baseline in the Raman window of 1800-2400 cm^{-1}. (b) SRS images of blank unloaded HeLa cells confirm the negligible cellular Raman background at 2129 cm^{-1} within the Raman-silent window. Scale bar: 20 μm.

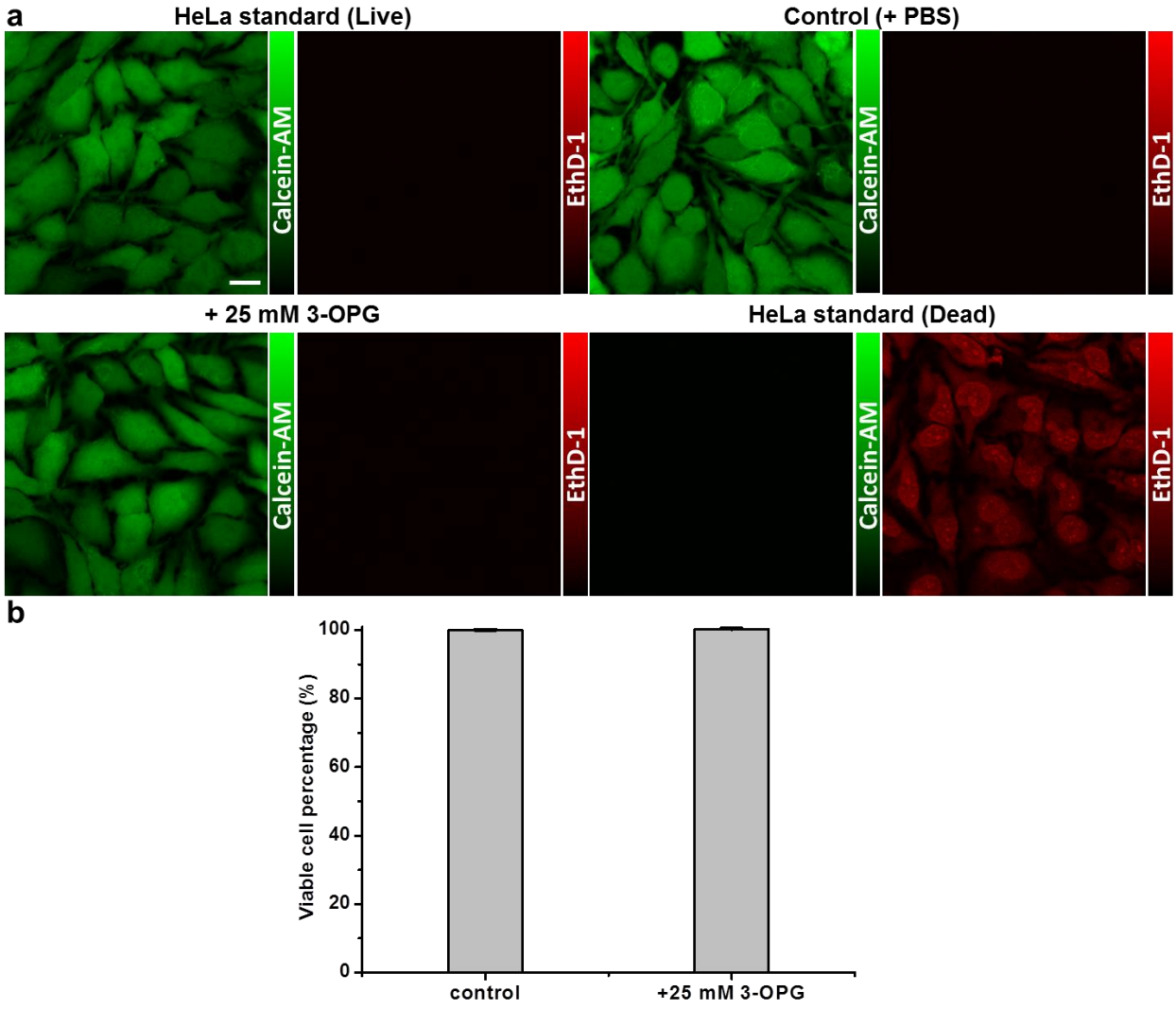


Figure S2. Cell viability test of mammalian cells incubated with 3-OPG. (a) Two-color fluorescence images of HeLa standards and HeLa cells incubated with or without 25 mM 3-OPG for 4 hours. Dead HeLa cell standard is obtained by treating live cells with 70% ethanol for 30 minutes. The assay is carried out using the LIVE/DEAD viability/cytotoxicity kit. Scale bar: 20 μm. (b) Quantification of cell viability with or without 3-OPG incubation. Data are shown as Mean + SD (n=3 for each group). No cytotoxicity of 3-OPG is observed in HeLa cells.

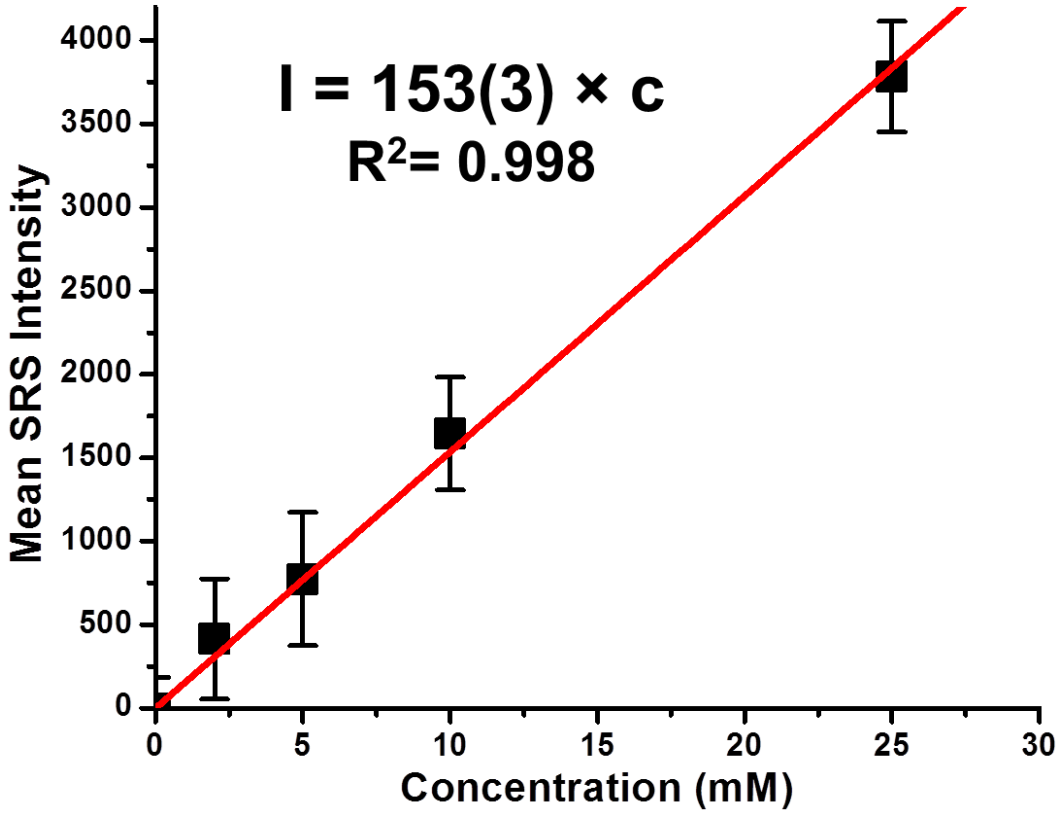


Figure S3. Linear correlation between measured SRS intensity at 2129 cm^{-1} and 3-O-propargyl-D-glucose PBS solutions of different concentrations with a detection limit (S/N=1) of ~1.4 mM based on the system noise.

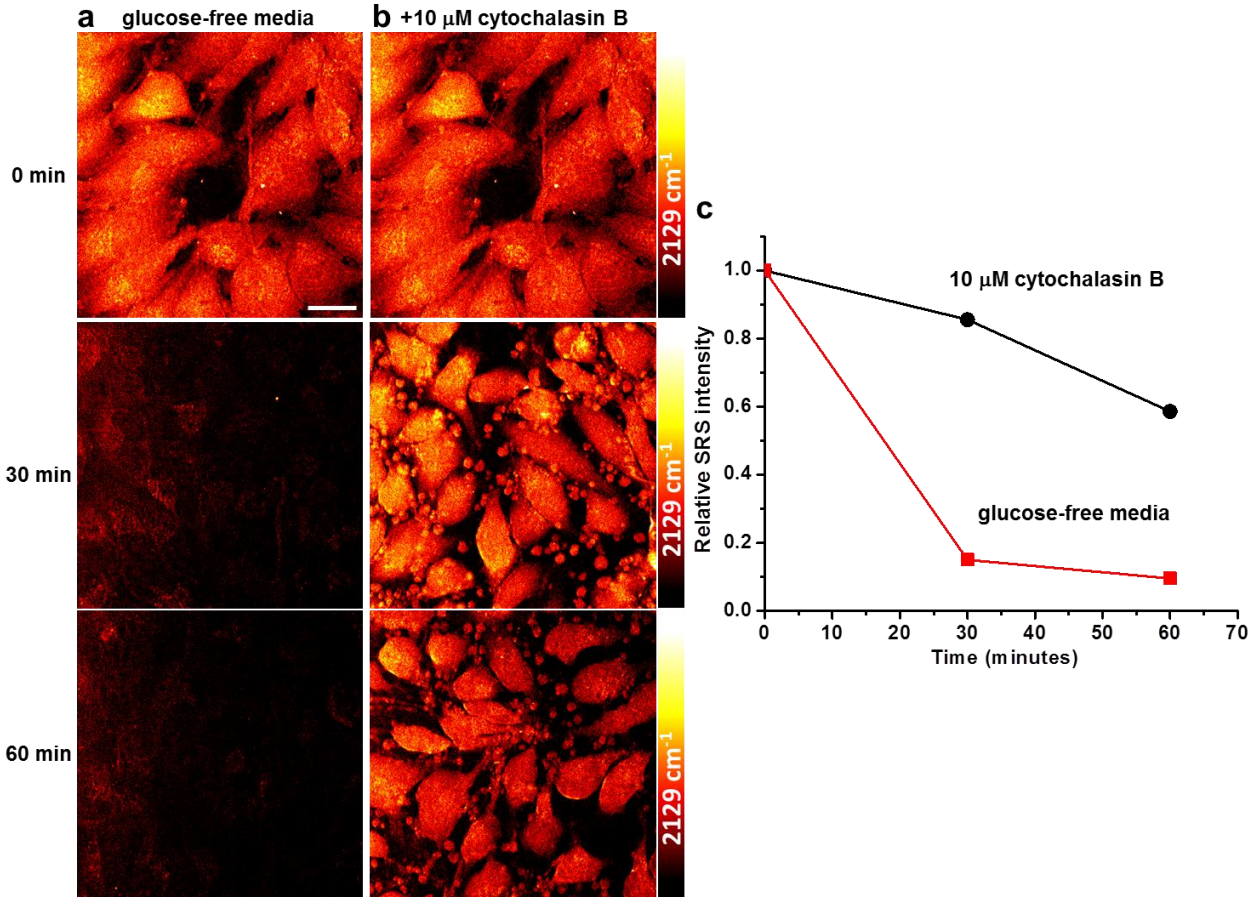


Figure S4. 3-OPG efflux experiments in live HeLa cells. (a, b) HeLa cells were incubated with 3-OPG at 37 °C before changing to glucose-free culture media without (a) or with (b) 10 μM cytochalasin B for 0, 30 and 60 minutes. Scale bar: 20 μm. (c) Quantification of 3-OPG intensity with time in both conditions.

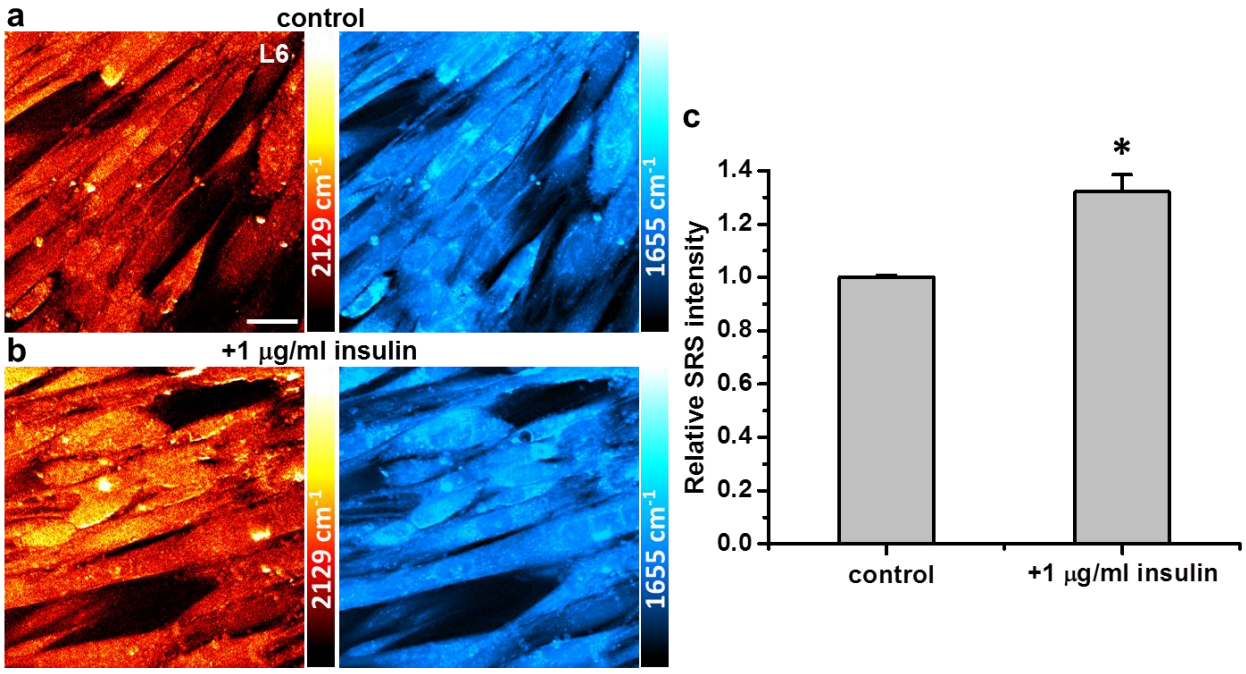


Figure S5. Insulin response of 3-OPG uptake in L6 muscle cells. (a, b) Representative SRS images of L6 rat muscle cells incubated with 3-OPG at 37 °C after treating with or without 1 μg/ml insulin for 24 hours. Scale bar: 20 μm. (c) Quantifications of 3-OPG uptake in both conditions. Data are shown as Mean + SD. *, $p<0.05$ by Student's *t*-test.

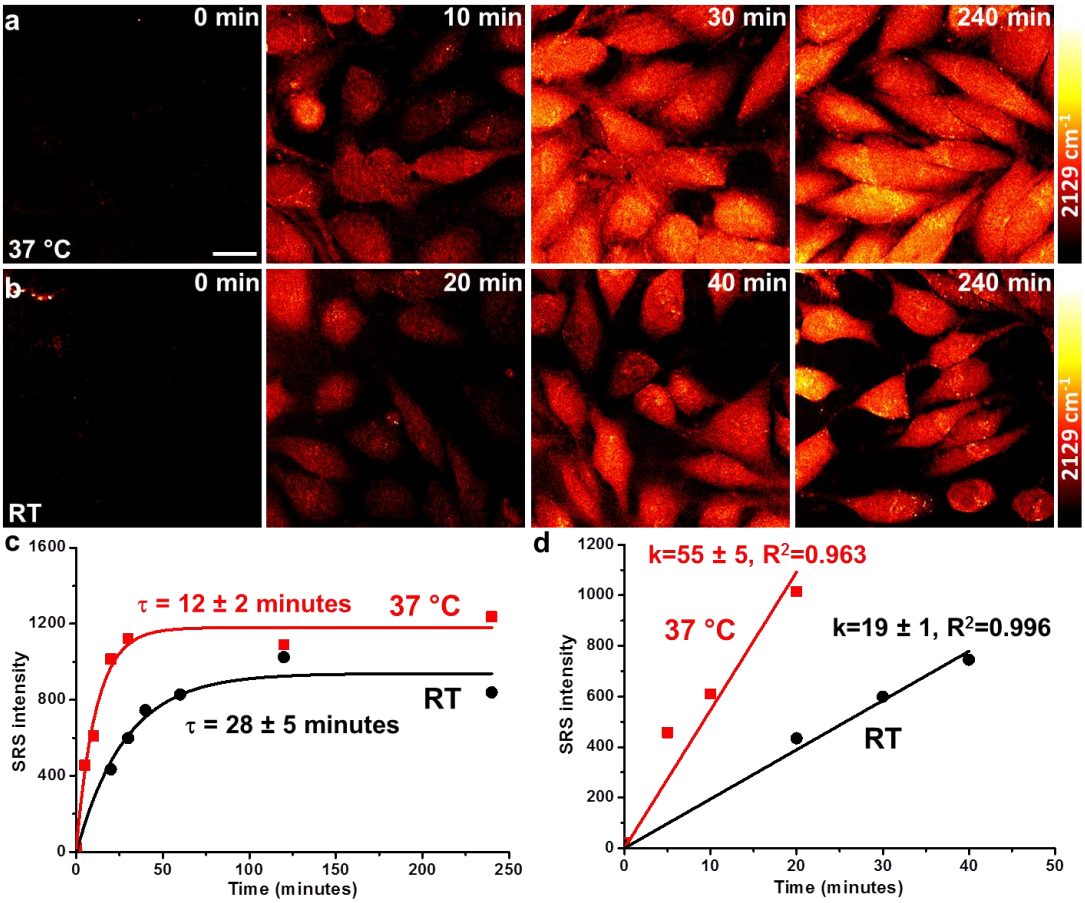


Figure S6. Uptake kinetics of 3-O-propargyl-D-glucose in live HeLa cells at 37 °C and room temperature. (a, b) Representative SRS images of HeLa cells incubated with 3-OPG at 37 °C (a) and RT (b) for various time durations before washing with PBS. Scale bar: 20 μm. (c) Single-exponential fitting of intracellular 3-OPG buildup with time at both conditions. (d) Linear fitting of initial rates of 3-OPG uptake at both temperatures. The fitting linearity improves at lower temperature.

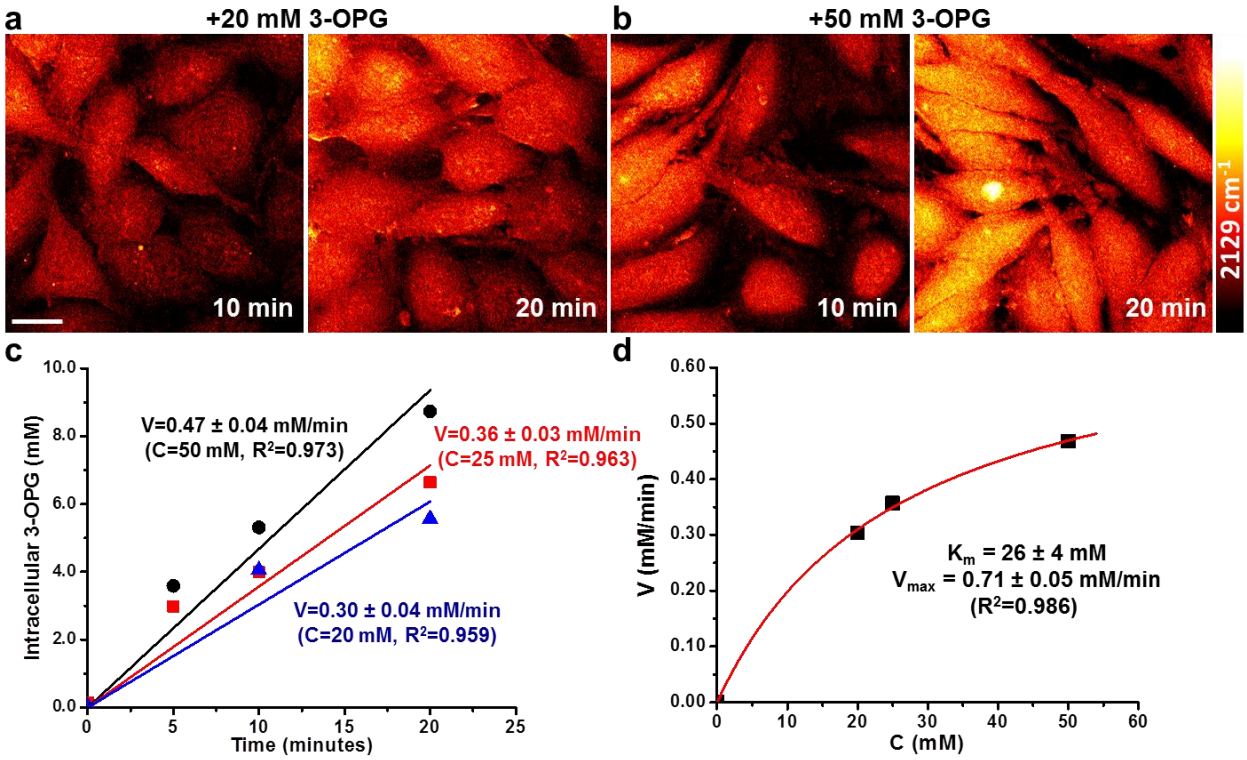


Figure S7. Analysis of uptake parameters of 3-O-propargyl-D-glucose (3-OPG) in live HeLa cells at different incubation concentrations. (a, b) Representative SRS images of HeLa cells incubated with 20 mM (a) and 50 mM (b) 3-OPG at 37 °C for short times (10 and 20 minutes). Scale bar: 20 μm. (c) Linear fitting of initial 3-OPG uptake rate at 20, 25 and 50 mM incubation concentrations. (d) Hill equation (n=1) fitting of initial uptake rates vs. 3-OPG concentrations gives K_m=26 mM and V_{max}=0.71 mM/min for HeLa glucose transporters. If assuming HeLa cells have dimensions of 20 μm×20 μm×10 μm, V_{max}=0.71 mM/min=0.28 nmol/10^5 cells/min.

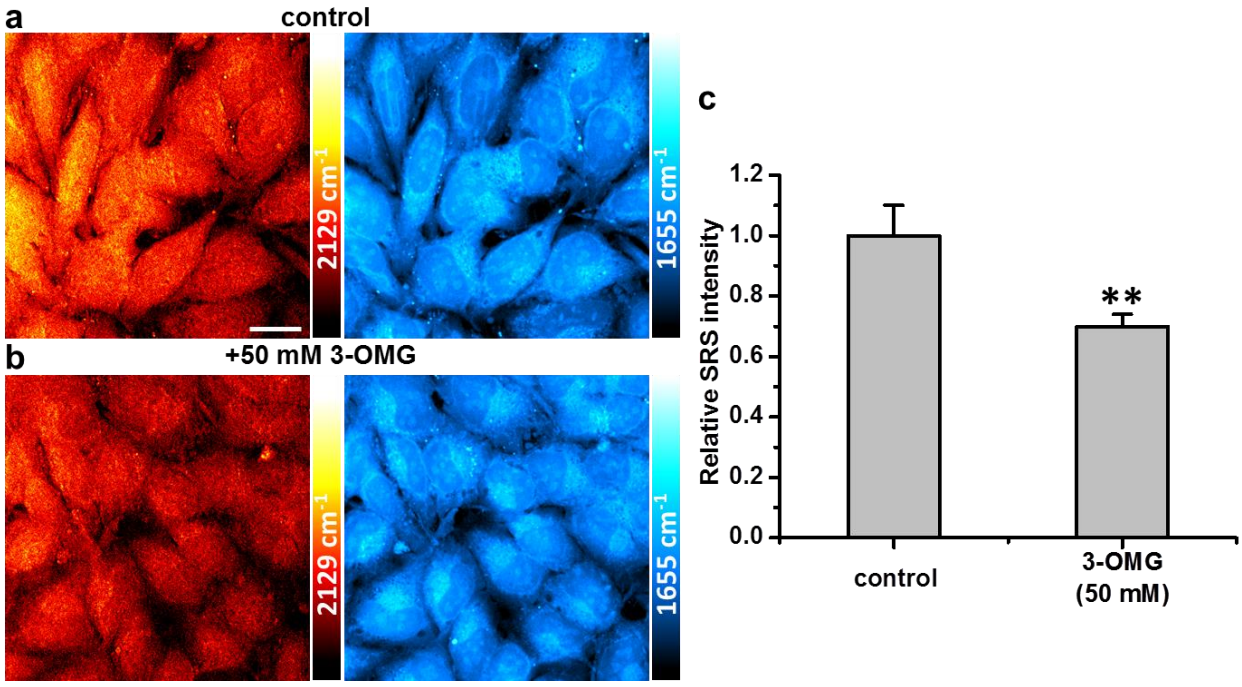


Figure S8. Competition experiment of 3-OPG uptake using non-metabolizable glucose analogue 3-OMG in live HeLa cells. (a, b) Representative SRS images of HeLa cells incubated with 3-OPG at 37 °C in the absence (a) or presence (b) of 50 mM 3-OMG. Scale bar: 20 μm. (c) Quantifications of 3-OPG uptake in both conditions. Data are shown as Mean + SD (n=3 for each group). **, $p<0.01$ by Student's *t*-test.

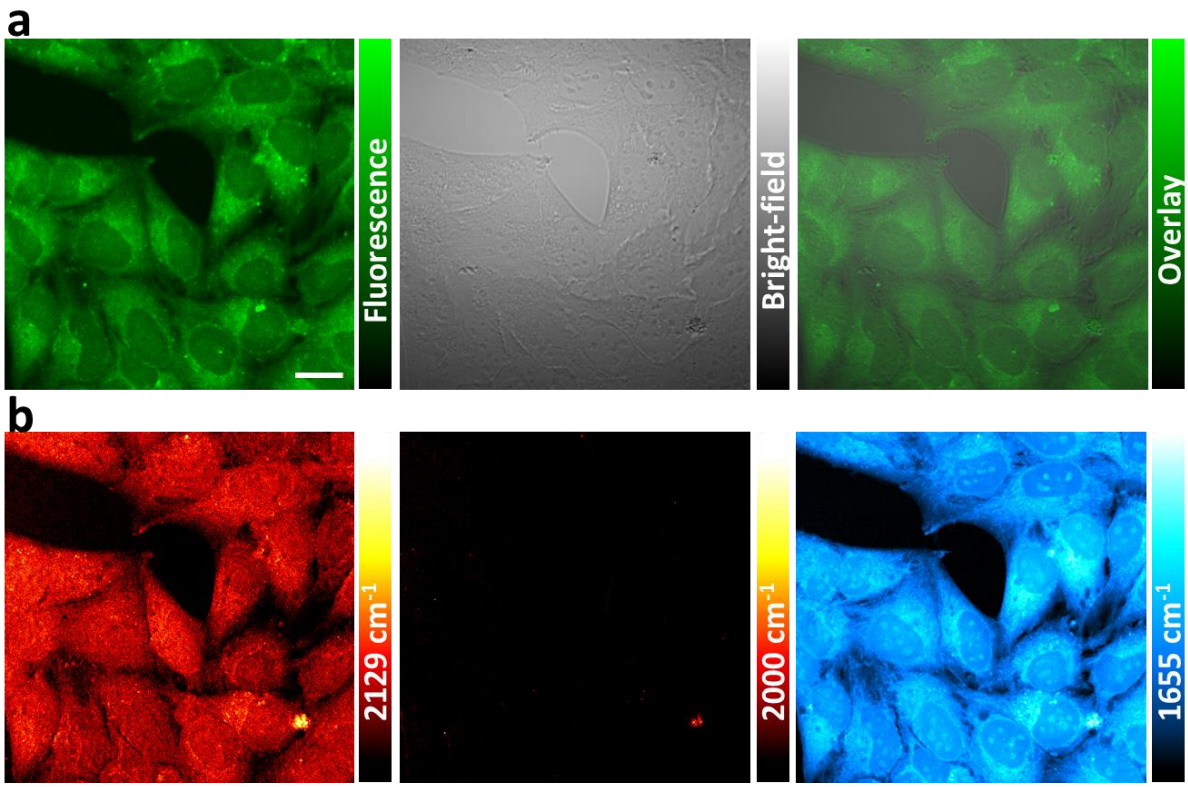


Figure S9. Simultaneous fluorescence and SRS imaging of live HeLa cells co-incubated with 2-NBDG and 3-O-propargyl-D-glucose. (a) Confocal fluorescence and bright-field images of live HeLa cells. (b) SRS images of live HeLa cells at three channels. All images display the same set of cells. Scale bar: 20 μm.

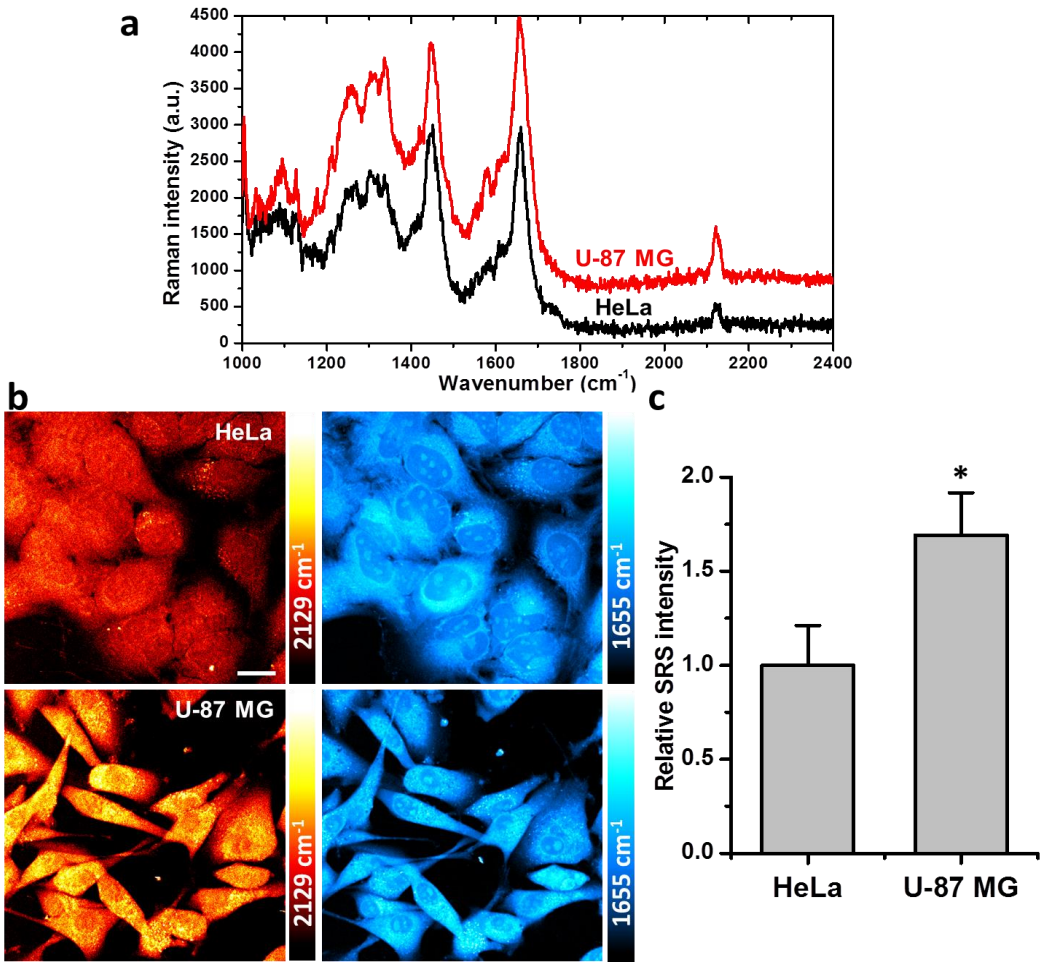


Figure S10. Comparison of 3-OPG uptake levels in cancer cells with different malignancy. (a) Spontaneous Raman spectra of HeLa and U-87 MG cells incubated with 3-OPG. (b) SRS images of 3-OPG distribution in live HeLa and U-87 MG cells. Scale bar: 20 μm. (c) Significantly enhanced uptake of 3-OPG in U-87 MG cells compared with HeLa cells. Data are shown as Mean + SD (n=3 for each group). *, $p<0.05$ by Student's *t*-test.